AD-A066 456 VIRGINIA POLYTECHNIC INST AND STATE UNIV BLACKSBURG --ETC F/G 6/6
THE USE OF DOUBLE TRANSLOCATIONS TO CONTROL POPULATIONS OF THE --ETC(U)
MAR 79 M H ROSS, D G COCHRAN N00014-77-C-0246

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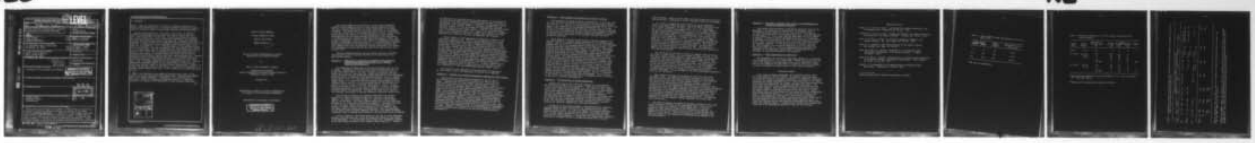

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(12) LEVEL II

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER (6) (9) Annual repto no. 2, | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
The Use of Double Translocations to Control Populations of the German Cockroach.

5. TYPE OF REPORT & PERIOD COVERED
Annual
3/1/78-2/28/79

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s) (10)
Mary H. Ross
Donald G. Cochran

8. CONTRACT OR GRANT NUMBER(s) (15)
N00014-77-C-0246

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Va. Polytechnic Inst. & State Univ.
Blacksburg, VA 24061

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (11) 19 Mar 79

11. CONTROLLING OFFICE NAME AND ADDRESS
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217

12. REPORT DATE

13. NUMBER OF PAGES

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) (12) 11 p.

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Distribution of this report is unlimited.

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Genetic control
Cockroach control
Translocation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The goal of this project is to conduct a "field" test of a unique type of sterility mechanism, embryonic trapping, for control of the German cockroach, Blattella germanica (L.). Trapping of viable embryos occurs when their number is reduced by genetic lethality to a point at which they are unable to force open the egg case at the time of hatch. Sterile egg cases occur frequently in matings of double translocation heterozygotes. Their frequency depends on the amount of lethality characteristic of the particular

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S/N 0102-LF-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. ABSTRACT

double. Using sex differences in lethality (chromosome disjunction) of certain single translocations, it has been possible to establish a crossing system for producing totally sterile double translocation-carrying males.

The project was planned on a 3-yr basis, with 2 years of laboratory research and development to be followed by a "field" test in the 3rd year. This report covers the 2nd year. The work was conducted according to 6 specific objectives. First, a population experiment with a freshly-collected field strain (USS Kennedy) was completed. Growth was suppressed following 3 monthly releases of an incompletely sterile double male, T(3;7;12). Under Obj. 2, analysis of a newly developed, totally sterile male, T(8;9), T(4;8;10), was completed. Obj. 3 involved expansion of the parental stocks, T(8;9) and T(4;8;10). This was accomplished, and intercrosses were initiated so as to supply double males of suitable age for a 1st release either the wk of April 2 or 9, 1979. Obj. 4 was aimed at establishing procedures for collection and analysis of shipboard populations which could be used to estimate the size of both a pre-treatment and a residual population. Treatment-collection was made possible through the help of Drs. McDonald and Egan at the Norfolk Naval Base. Statistical analysis was worked out by Mr. Keil. Objs. 5 and 6 involved arrangements for an experimental ship, for 2 treatment-collections (successive weeks), and for other details of initiating the field trial that are largely in the hands of Navy entomologists. Overall, the 6 objectives for this past year had one goal - to place us in readiness to conduct a well-designed field trial, to be initiated in late March-early April of 1979.

One of the important points emerging from this year's study is that releases of sterile males may be very small indeed - certainly much smaller than a pre-cleanout population. Nevertheless, the sterile males are potentially capable of suppressing population growth or, if completely successful, of leading to the elimination of small residual populations.

OFFICE OF NAVAL RESEARCH

Contract #N00014-77C-0246

Task No. NR 205-028

ANNUAL REPORT NO. 2

The Use of Double Translocations to Control Populations of the German Cockroach

by

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19 March 1979

This report summarizes the results of the 2nd year of laboratory research and development carried out under Contract #N00014-77C-0246, "The Use of Double Translocations to Control Populations of the German Cockroach". The overall goal for this year was to place us in readiness to initiate a field test in late March-early April of 1979. The particular double selected for testing is the most promising of several double translocations that have been developed and studied in our laboratories. It is heterozygous for a typical translocation heterozygote, T(8;9), and an unusual interchange, T(4;8;10), that involves a progressive interchange between 3 chromosomes (Cochran and Ross 1977). The double males, selected from crosses between the two translocations, are fully competitive and cause complete sterility from embryonic trapping (inability of low numbers of living embryos to force open the egg case at the time of hatch, Keil and Ross 1977).

The work accomplished during the past Contract period is described below according to the 6 specific objectives that were listed in the Continuation Proposal.

Objective 1 - Completion of a population experiment using releases of double-translocation carrying males into a freshly-collected field population.

This experiment was begun late in the period covered by the 1st year of support. Procedures and partial results were described under Obj. 4b of the first Annual Report. In brief, releases of a partially sterile double male, T(3;7;12), were made into a population developed from cockroaches collected from the USS Kennedy, kindly forwarded to us by LCDR Lambdin. Males were placed in competition for females maturing in each of 3 nymphal groups (Table 1, col. 1). The designation "B" indicates a field-collected nymphal group, "A-1" stands for the first nymphal group produced by field-collected adults, and "A-2" for the 2nd group from the same parental set. Ratios of T(3;7;12) males to wild-type ranged from ca. 6-10:1. It was planned originally to reach a 10:1 for all releases. However, the sudden, synchronous production of a large group of nymphs (A-1) by the 20 field-collected females was unexpected. We suspect the shift from a field to the laboratory environment in some way stimulated oviposition and/or hatch.

Oothecal-bearing females were removed periodically from the main population in order to determine mating type, sterility, and progeny numbers. The results are shown in Table 2. There was no hatch beyond that of 3rd egg cases. All group B females mated with T(3;7;12) males and, probably due to chance alone, sterility was complete. Cytogenetic analyses of T(3;7;12) males showed a mean egg case sterility of 89% (Ross and Cochran, in press), similar to that in group A-1 and A-2 females. Only one female (group A-1) mated with a wild-type male. Thus the replacement of each target group by progeny numbering less than ½ the number of parents represented close to maximum effectiveness of this particular genetic mechanism.

Since all females of the target groups, B, A-1, and A-2, were eventually removed, only males were left in the main population during the last months of the experiment. In the 6th month, only 13 were left, presumably survivors of the last release and males of the youngest target group (A-2). Although

the population was separated during the later months, it was possible to use data from the female counts, separated progeny groups, and counts of cockroaches in the main population to reconstruct the population to show the general trend in growth (Table 3). Releases increased the total number by a factor of 2X to 4X, although if unchecked this population would have been double the total number (Table 3, total 2) by the 4th month, with a 7-fold difference by mo 5, etc. Death of the majority of released males by mo 6 heralded the decline in numbers that resulted in a total population less than that with wich the experiment was initiated.

Several aspects of this experiment are important in respect to the field trial; others are not. This is the 2nd laboratory test in which laboratory translocation-strain males competed successfully with field strain males (Ross 1978), this time in a population situation. In the present data, the occurrence of only one wild-type mating out of a total of 174 leaves no doubt that T(3;7;12) males were the more competitive of the two types. It is also noteworthy that this incompletely sterile double achieved population suppression through releases into only one generation of progeny. By analogy, a similar experiment using the completely sterile T(8;9),T(4;8;10) males would have brought the population close to extinction. These findings auger well for the field test. On the other hand, numbers released and their effect on total population numbers cannot be meaningfully compared to those of a field experiment, especially one following a preliminary population recution (see Obj.4). To have a population with 100 females maturing within a monthly interval, we would have to deal with infestations of 1000-1500 cockroaches (Sherron, unpubl. data)!

The results of this experiment are being prepared for publication. We will forward a copy of the manuscript as soon as it is completed.

Objective 2 - Analysis of the double translocation selected for field testing

This objective was also partially completed in the 1st year of this Contract, and all studies were finished by the time of the 6 mo report. Complete sterility from trapping occurred in 46 matings of T(8;9),T(4;8;10) males to wild-type (VPI strain) females. Counts of alternate (viable) vs adjacent (lethal) chromosome orientations in meiotic cells of T(8;9),T(4;8;10) male nymphs showed the typical disjunction properties of T(8;9) and T(4;8;10) were maintained in cells of the double males. This produced a high lethality (81%) and accounted for complete sterility from trapping (Keil and Ross 1978). A second lethality estimate was made by counting the numbers of viable (fully developed) embryos vs incompletely developed embryos in mature oothecae of wild-type females mated to double males. The results agreed closely with those from the chromosome analyses, showing an average embryonic lethality of 82%. We noted previously that two multivalents occur in the meiotic cells, rather than the ring-of-eight expected if the two translocations had chromosome 8 in common. However, we will continue to use the designation "T(4;8;10)" until we have definitive evidence as to the correct identification of chromosome "8".

Objective 3 - Stock expansion and production of males for release

The procedures for producing double translocations, in this case both T(3;7;12) and T(8;9),T(4;8;10), were outlined in the Continuation Request for 1978-79, p. 3. The first to be developed and expanded was for T(3;7;12). However, with completion of the population experiment (Obj. 1) and the choice of the newer double, T(8;9),T(4;8;10), for field testing, production of T(3;7;12) was discontinued, and the parental stocks reduced to a minimum. In their place, the T(8;9) and T(4;8;10) stocks were expanded as rapidly as possible within limitations of the available personnel and their biological properties.

Saving females of the T(8;9) and T(4;8;10) crossing systems for 2nd egg cases enabled us to double nymphal production by late December-early January. One set of T(8;9)♂ X T(4;8;10)♀ crosses was set up in mid-December, although full use of the expanded parental system came in January. First hatch of intercross progeny occurred the 1st wk of January. From these, 24 double males were selected early in March. By late March, we estimate selection of at least 100-150 double males/wk. Saving all double males produced during March will give a heterogeneous group ranging from 4th instar to 2-wk-old adults available for a 1st monthly release in the 1st or 2nd wk of April. This should provide males of suitable age to compete for females maturing within the monthly interval between releases. Only part of the large group of males we expect to select late in March would be used in the 1st release - the others would be saved to form the older adult contingent of the 2nd release. We are trying to maintain as much flexibility in the system as possible. We are taking into account that the 1st releases, aimed only at large nymphs left following a treatment program, will be small, but that increased numbers will be needed to meet nymphal production from previously mated females. We will, if necessary, be able to shift to a larger vessel than a minesweeper. Alternatively, if two smaller ships were available during overlapping periods, we should be able to do 2 releases simultaneously.

Objective 4 - Characteristics of natural populations

Samples from several Navy ships were analyzed as to age structure and the results covered in the 6 mo report. These were of primary value in that they showed late instar nymphs, i.e., the target for the releases, tend to form a rather small segment of the population, as in other analyses of cockroach populations (Ross and Wright 1977; Sherron, unpubl.). These data are not repeated here as the lack of a standardized collection procedure made them unsuitable for statistical analyses. The primary need for this project was to develop a method for estimating population size both before and after insecticide treatment, as has been worked out below by Mr. Keil through the cooperation of Drs. McDonald and Egan who made possible the requisite treatment and collection procedures.

Preliminary to the release program we conducted population estimation exercises aboard two Naval vessles with the cooperation of Drs. Egan, McDonald and their staff. On 16 Nov. we sampled the cockroach population aboard the USS Yarnell in the main galley spaces. The sample was taken with a vacuum cleaner equipped with an in-line collection device during a routine pest control operation. Cockroaches were flushed with a pyrethrin spray over a 1% Baygon® barrier or flushed with Baygon® and collected

with the vacuum. Before a second sample could be obtained the USS Yarnell departed Norfolk. Consequently, the 1st collection could not be analyzed.

On 17 Nov. we sampled the ward room mess of the USS Guadacanal (LPH7) in the manner described above. The first sample contained a total of 113 German cockroaches: 41 adults (18 ♂, 23 ♀), 31 large nymphs (17 ♂, 14 ♀), 24 medium sized nymphs (12 ♂, 12 ♀) and 17 small nymphs. Thirteen oothecae were collected with a mean of 43.15 nymphs per ootheca. The same spaces were sampled on the Guadacanal on 1 Dec. We captured a total of 16 cockroaches, 5 of which had hatched in the interim. The contents of this sample were: 8 adults (5 ♀, 3 ♂), one large female nymph and 2 mid sized nymphs (1 ♂, 1 ♀). This indicates a catchability of 91.13%. A third sample would be predicted to yield approximately one cockroach if this catchability is relatively constant. This indicates a total pretreatment population of 129 cockroaches. We found three oothecae with an average of 37.33 embryos in each. The proportion of females with ootheca remained about the same, 60% and 56.52%, between the first and second samples.

The second ship we were able to obtain two samples from was the USS San Diego, a supply vessel. The first sample was taken on 30 Nov. in the main galley where we found moderate to heavy populations, LCDR McDonald's estimation. The second collection was made on 14 December by Dr. Egan and was talleyed only with regard to total numbers. Our analysis of the population was thus limited total population size. The total captured in the first sample was 276, 128 adults (58 ♂, 70 ♀) and 148 nymphs. The second catch was comprised of 37 individuals. This is indicative of a catchability of 88.18%. An estimated third sample would be five residual cockroaches. Here an estimate of the total pretreatment population would be 329 cockroaches.

These analyses are based on the method of Carle and Strub (1978) and Zippin (1956). The population estimates are based on removal data obtained from successive and discrete capture periods with the assumption that catchability will remain constant between captures. As we can distinguish cockroaches entering the population between captures by natality they can be eliminated from consideration in this method. With catchabilities this high, approaching 90%, this method of population estimation documents how close the chemical treatment approaches total elimination of the population in the treated spaces and gives us an unbiased estimate of the pretreatment population with associated confidence intervals. Thus it is possible to evaluate the efficacy of the sterile male release by comparing pretreatment and post release population levels. An additional advantage is that this method is easily integrated into routine pest control activities.

The above establishes the method for estimating populations both prior to and following a treatment on the experimental ship. Since a first release is aimed only at medium to large nymphs left in a residual population (those expected to mature in mo following 1st release), it appears very few will be needed to reach a 10T,T,:1 wild-type male ratio, probably no more than 50. The 2nd and especially the 3rd releases will need to be larger to meet production by previously-mated females of the residual population.

Objective 5 - Selection of release sites, control, and arrangements for preliminary population reduction

These matters were discussed in some detail during the visit of CDR Mulrennan to our laboratory in January 1978, the subsequent visit of LCDR Lambdin and his technician, and the June visit of Dr. Cochran, Dr. Ross, and Clifford Keil to the Norfolk Naval Base. With the departure of LCDR Lambdin, Lt. (j.g.) Peter Egan was appointed our liaison officer at Norfolk, in charge of making arrangements for both 2 or, preferably, 3 treatments with collections by Mr. Keil, to be followed by a 1st release within 2 wks. Sites of release are to be "hot spots" identified during the treatment procedure. It was decided the best "control" would be Mr. Keil's estimate of the original population, using the statistical tests noted under Obj. 4. It was our understanding a minesweeper was available for the experiment, which seemed to be preferable to the destroyer, as originally proposed for the experiment.

Objective 6 - Other preparations for field tests

The arrangements for getting the experiment underway are in the hands of LCDR McDonald and Lt. (j.g.) Peter Egan. Through their cooperation and that of the skipper of the experimental ship, we are optimistic a field test will be conducted that will serve as a model for any future attempts to control cockroaches by sterile males or other genetic mechanisms.

Concluding Remarks

First trials of genetic mechanisms for controlling various insect pests have frequently met with failure. The primary reasons have been: (1) insufficient study of the genetic mechanism, (2) inadequate information concerning the ecology and dynamics of the target population, or (3) underestimating the numbers needed for release. We have taken these possibilities into account and prepared for the present experiment accordingly. The double males have been placed in competition with field strain males for field strain females. They tended to out-compete the field strain males. The females showed no preference for males of the same strain. Likely aggregation areas can be identified with the expertise of Navy entomologists such as LCDR McDonald and/or Lt. (j.g.) Egan of the Norfolk Naval Base. Thanks to the statistical training of Mr. Clifford Keil, procedures have been established that will give a statistically reliable indication of both the population prior to and following routine chemical control. A system for producing sterile males has been established that should ensure a more than adequate supply. It remains only to proceed with treatment and collection on the experimental ship and to make the first release.

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*Papers prepared and/or published during past 12 months.

Table 1 - Target nymphal groups and releases of T(3;7;12) males (T,T).

Target group		Number released	Approximate T,T:+/+ male ratios
Symbol[a]	Number		
B	16	70	10:1
A-1	355	769	6-7:1
A-2	211	651	9-10:1

[a] See text for explanation.

Table 2 - Effects of releases of T(3;7;12) males on mating types and progeny numbers.

Target group	Mating types[a]	Sterility of 1st egg cases (%)	Hatch			
			1st egg case	2nd egg case	3rd egg case	Total
B (16)	4 T,T	100	0	0	0	0
A-1 (355)	100 T,T	94	25	18	0	
	3 T,T[b]	0	21	14	3	
	1 +/+	0	29	25	13	
		90 avg	75	57	16	148
A-2 (211)	64 T,T	92	22	11	0	
	2 T,T[b]	0	19	19	5	
		89 avg	41	30	5	76

[a] Determined from examination of mature oothecae. T,T - T(3;7;12) mating; +/+ - wild-type mating.

[b] Oothecae with low embryonic lethalities (54-58%).

Table 3 - Growth of a recently-collected field strain population following sequential releases of T(3;7;12) males (double translocation heterozygotes).

Age[a]	Progeny groups and numbers in the population[b]						
	Mo 1	Mo 2	Mo 3	Mo 4	Mo 5	Mo 6	Mo 7
Ad	A: 33	A,B: 25	B,A-1: 212	A-1,A-2: 292	A-1,A-2: 242	A-1,A-2, A-1-1: 201	A-1,A-2,A-1-1, A-1-2,A-2-1: 173
Md-Lg	B: 16	A-1: 295	A-2: 175		A-1-1: 62	A-1-2, A-2-1: 81	A-1-3,A-2-2: 38
Sm	A-1: 355	A-2: 211		A-1-1: 75	A-1-2, A-2-1: 98	A-1-3, A-2-2: 46	A-2-3: 5
Total: (1)	404	531	387	367	402	328	216
(2)	474	1360	1680	1464	1127	459	216
	(rel. #1)	(rel. #2)	(rel. #3)				

[a] Age is indicated by "ad" for adults, "Md-Lg" for medium to large-sized nymphs (3rd-6th instars), and "Sm" for small nymphs (1st-2nd instars).

[b] Symbols for progeny groups are explained in the text. Nymphal survival in wild type groups is estimated at 83% (Ross 1977). Adult wild-type estimates are based on counts of oothecal-bearing females and an assumption ca. 10% of the adult males were wild-type (see text for details).